

The Enthalpies of Combustion and Formation of Cholesterol [cholest-5-en-3-ol (3β)]

Walter H. Johnson

Institute for Materials Research, National Bureau of Standards, Washington, D.C. 20234

(March 6, 1975)

The enthalpy of combustion of cholesterol was measured in an adiabatic, rotating-bomb calorimeter capable of high precision with relatively small samples. The random error for the experimental measurements was 0.006 percent which may be compared with approximately 0.3 percent for prior investigations on this substance. The results obtained for the enthalpy of combustion and the derived enthalpy of formation together with the estimated overall uncertainties are:

$$\begin{aligned}\Delta H_c^\circ(25^\circ\text{C}) &= -16524.0 \pm 3.9 \text{ kJ/mol.} \\ \Delta H_f^\circ(25^\circ\text{C}) &= -674.8 \pm 4.1 \text{ kJ/mol.}\end{aligned}$$

The results of prior investigations are discussed briefly.

Key words: Cholesterol; clinical standard; combustion, heat of; enthalpy; formation, heat of; reference material.

1. Introduction

Cholesterol [cholest-5-en-3-ol (3β)] is one of the basic sterols which occurs in all animal tissues and is of great importance to animal life. The thermodynamic properties of this material are, therefore, important in the study of the biochemical processes in which it is involved. It is one of the biomedical chemicals which is certified for purity and offered as a standard reference material (SRM 911a) by the NBS Office of Standard Reference Materials. Since this material is of natural origin it is expected to be essentially free of sterol isomers.

Prior investigations on the enthalpy of combustion of this compound have been subject to relatively large uncertainties which may be due to the difficulty in obtaining a highly purified sample.

2. Materials

Benzoic Acid

The benzoic acid used for calibration of the calorimeter was SRM 39i for which the energy of combustion under certificate conditions was certified to be 26434 J/g. Correction to standard conditions at 28 °C gave $\Delta U_c^\circ(28^\circ\text{C}) = -26410.36 \text{ J/g}$.

Oxygen

High-purity oxygen was used for both calibration

and combustion experiments; the main impurities being 3 ppm of nitrogen, 2 ppm of water, and 0.8 ppm of nitrous oxide. The presence of these impurities would not have a significant effect upon the results of this investigation.

Cholesterol, NBS Standard Reference Material 911a.

The results of the analytical procedures are given in the NBS Certificate of Analysis [1]¹ and some of these procedures are summarized here for convenience.

The melting point of this material, measured in a sealed tube under vacuum, is 149.0 to 149.4 °C. The specific rotation $[\alpha]_D^{20}$ is $-0.700 \text{ rad } (-40.1^\circ)$ (*c*, 1; CHCl_3). Microchemical analysis found carbon 83.86 ± 0.05 and hydrogen 12.00 ± 0.05 weight percent; the theoretical percentages based on $\text{C}_{27}\text{H}_{46}\text{O}$ are 83.87 and 11.99, respectively.

Heating the material at 100 °C and 3 Pa (0.02 torr) resulted in a nearly constant loss in weight of 0.01 percent per hour over a 36 hour period; such a volatilization may be related to degradation and/or sublimation processes. These results appear to eliminate the probability of water as an impurity. The certified purity of this sample is given on the Certificate of Analysis as 99.8 ± 0.1 percent.

¹ Figures in brackets indicate the literature references at the end of this paper.

3. Apparatus and Procedure

The platinum-lined bomb and the adiabatic calorimeter have been described [2]. The volume of the bomb is 98 cm³.

The sample was stored in a desiccator over magnesium perchlorate and kept at approximately 0 °C. The desiccator was warmed to room temperature before opening. Samples of approximately 0.18 g each were pressed into pellets. The sample was weighed into a platinum crucible which was placed in the bomb so that the pellet was in contact with a 2-cm length of 0.075-mm diam platinum wire connected between the electrodes. By means of a micro-buret, 0.300 cm³ of water was added to the bomb which was then sealed.

The bomb was purged of nitrogen by filling with oxygen to 30 atm,² releasing the gas and refilling to 31.62 atm. The bomb was transferred to the calorimeter, the temperature of filling was determined and the bomb was heated electrically to 24.98 °C. The calorimeter jacket was evacuated, the adiabatic temperature controls placed in operation and the system left over-night.

On the following morning the pressure in the calorimeter jacket had been reduced to about 4.0×10^{-6} Pa. Calorimeter temperatures were observed at intervals during a 30-min initial rating period. An 18,000 μ F condenser was charged to 24.5 V and discharged through the platinum wire which melted and ignited the sample. The quantity of electrical energy introduced for ignition was calculated from the drop in potential across the condenser and corrected for the portion dissipated in the connecting leads. It was not necessary to rotate the bomb during these experiments. When thermal equilibrium was established, calorimeter temperatures were observed at intervals during a 40-min final rating period. The observed temperature rise was obtained by extrapolation of the time-temperature curves, for the initial and final rating periods, to the actual time of ignition. A drift of approximately 0.001 °C/h was observed during the rating periods; this was due to the heating effect of the continuous 5 mA current through the platinum resistance thermometer.

The bomb was removed from the calorimeter assembly, the gaseous contents released and the bomb solution transferred to a glass beaker. The solution was boiled to expel carbon dioxide, cooled and titrated with standard alkali to determine the quantity of nitric acid produced from traces of nitrogen in the bomb atmosphere.

4. Results and Calculations

The results of the benzoic acid calibration experiments are given in table 1. All calculations including correction to standard conditions [3] were performed by computer with the tabulated values taken directly from the print-out and rounded for convenience. The

headings, in the order in which they appear in the tables are described as follows:

EEE-Std, the effective energy equivalent of the standard, empty calorimeter;

Cv-cont(i), the heat capacity of the initial bomb contents, including the sample, crucible, water and oxygen;

Corr to tm, a correction to the heat capacity of the initial system for deviation of the actual mean temperature of the experiment from the standard mean temperature;

Corr-parts, a correction for alterations to the system during the series of experiments.

EEE-actual, the effective energy equivalent of the actual system at the actual mean temperature of the experiment;

Temp. rise, the observed increase in temperature of the system following ignition of the sample;

Q-total, the total quantity of energy evolved;

q-ign, the electrical energy added to the system to ignite the sample;

q-decomp HNO₃, the calculated quantity of energy required to decompose any nitric acid, formed in the combustion process, into nitrogen, oxygen and water;

q-WC, the Washburn Correction [3] applied to convert all reactants and products to their respective standard states at the actual final temperature;

q-corr to std t_f , a correction applied for deviation of the actual final temperature to the selected standard final temperature;

Q-std react, the energy evolved by the reaction with products and reactants in their respective standard states at the selected final temperature;

m_s , the mass of sample;

ΔU_c° , the energy evolved by the standard reaction at the standard final temperature in J·g⁻¹.

The following values were used in the calculations:

	Density, g·cm ⁻³	Cp, J·g ⁻¹ ·K ⁻¹	$\left(\frac{\partial U}{\partial P}\right)_T$ J·g ⁻¹ ·atm ⁻¹
Benzoic acid.....	1.320	1.21	-0.0125
Cholesterol.....	1.067	1.46	-0.012

The density of cholesterol was taken from the Handbook of Chemistry and Physics [5]. The values taken for the heat capacity and the compressibility coefficients were estimated.

Other auxiliary data were taken from Wagman et al. [4]. The unit of energy is the joule; for conversion from the conventional thermo-chemical calorie, 1 calorie has been taken as equivalent to 4.1840 J.

For the calibration experiments, the value of EEE-Std was calculated by means of an iterative procedure from the mass of sample. Due to rounding of the individual items from the more exact values in the computer print-out, small differences may result by calculation from the tabulated data.

The results of the combustion experiments on

² 1 atm = 101325 Pa.

TABLE 1. *Results of the calibration experiments with benzoic acid*

Expt. No.	495	524	525	526	527	529
EEE-Std (26.5°C).....J/K...	2519.46	2519.63	2519.47	2519.70	2519.68	2519.46
Cv-cont(i).....J/K...	4.82	4.81	4.72	4.73	4.70	4.74
Corr to tm.....J/K...	0.08	0.11	0.15	0.09	0.10	0.14
Corr-parts.....J/K...	-0.16	0.00	0.00	0.00	0.00	0.00
EEE-actual.....J/K...	2524.20	2524.55	2524.34	2524.52	2524.48	2524.34
Temp rise.....K.....	3.133364	3.108335	3.159280	3.089474	3.100061	3.173467
Q-total.....J.....	-7909.24	-7847.16	-7975.09	-7799.44	-7826.05	-8010.91
q-ign.....J.....	1.22	1.06	1.18	0.79	1.11	1.16
q-decomp HNO ₃J.....	0.70	0.21	0.30	0.47	0.17	0.84
q-WC.....J.....	6.26	6.20	6.29	6.16	6.13	6.36
q-corr to 28°C.....J.....	-0.04	-0.04	-0.06	-0.03	-0.04	-0.06
Q-std react.....J.....	-7901.10	-7839.73	-7967.38	-7792.05	-7818.67	-8002.62
m _gg.....	0.299167	0.296843	0.301676	0.295038	0.296046	0.303011
ΔU_c° (28°C).....J/g...	-26410.36	-26410.36	-26410.36	-26410.36	-26410.36	-26410.36
Mean, EEE-std (26.5°C).....J/K...	2519.56					
sdm.....J/K...	0.048					

TABLE 2. *Results of the cholesterol combustion experiments*

Expt. No.	517	518	519	520	521	522
EEE-Std (26.5°C).....J/K...	2519.56	2519.56	2519.56	2519.56	2519.56	2519.56
Cv-cont(i).....J/K...	4.70	4.71	4.70	4.72	4.72	4.70
Corr to tm.....J/K...	0.06	0.07	-0.06	0.01	0.01	-0.01
Corr-parts.....J/K...	0.00	0.00	0.00	0.00	0.00	0.00
EEE-actual.....J/K...	2524.32	2524.34	2524.20	2524.29	2524.29	2524.25
Temp rise.....K.....	3.052085	3.061326	2.923671	3.006016	3.000576	2.975157
Q-total.....J.....	-7704.44	-7727.83	-7379.93	-7588.06	-7574.32	-7510.04
q-ign.....J.....	0.99	0.89	0.89	1.04	1.04	0.89
q-decomp HNO ₃J.....	0.13	0.42	0.42	0.15	0.19	0.20
q-WC.....J.....	2.90	2.91	2.76	2.86	2.86	2.81
q-corr to 28°C.....J.....	-0.03	-0.04	0.04	0.00	0.00	0.01
Q-std react.....J.....	-7700.45	-7723.65	-7375.82	-7584.01	-7570.23	-7506.13
m _gg.....	0.180520	0.181056	0.172901	0.177799	0.177491	0.175977
ΔU_c° (28°C).....J/g...	-42657.04	-42658.90	-42659.21	-42654.96	-42651.35	-42654.04
Mean.....J/g...	-42655.92					
sdm.....J/g...	1.24					

cholesterol 911a are given in table 2. The results are summarized in table 3.

The uncertainties assigned to the values ΔU_c° and ΔH_c° were obtained by the combination of 2 sdm for the calibration experiments with 2 sdm for the combustion experiments, 0.01 percent for the uncertainty in the certified value for benzoic acid and 0.02 percent for the effects of possible impurities in the sample.

The uncertainty of 0.02 percent, assigned to the possible effect of impurities in the sample was obtained by assuming the impurity to consist of a similar organic substance for which the heat of combustion differed by 70 percent.

The uncertainty assigned to ΔH_f° was obtained by the combination of the uncertainty in ΔH_c° with 0.01 percent for the uncertainty in the heat of formation of carbon dioxide and 0.01 percent for the uncertainty in the heat of formation of water.

The results given in tables 2 and 3 correspond to the process:

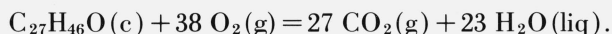


TABLE 3. Summary of the results of this investigation

ΔU_c° (28°C) = - 42655.9 ± 10.0 J/g
ΔU_c° (28°C) = - 16493.3 ± 3.9 kJ/mol
ΔH_c° (28°C) = - 16520.9 ± 3.9 kJ/mol
ΔH_c° (25°C) = - 16524.0 ± 3.9 kJ/mol
ΔH_f° (25°C) = - 674.8 ± 4.1 kJ/mol

5. Comparison With Other Reported Values

Berthelot and Andre [6] purified a commercial sample by digestion on a steam bath with calcium hydroxide and water. The cholesterol was taken up in ether, the ether was evaporated and the residue recrystallized from alcohol. The process was repeated until the elemental analysis showed the correct results. They performed two combustion experiments. Correction of their results to modern units and standard conditions yields -16685 ± 166 kJ/mol (-3987.9 ± 40 kcal/mol) for ΔH_c° (25°C).

Bills, Cox, and Steel [7] removed ergosterol from a sample of cholesterol by succession bromination and

debromination. The sample was recrystallized from alcohol and dehydrated for 15 h at 80 °C in high vacuum. They performed three combustion experiments with a precision of 0.3 percent. Correction of their data to current units yields -16657 ± 50 kJ/mol (-3981.1 ± 12.0 kcal/mol) for ΔH_c° .

Paoli, Garrigues, and Patin [8] checked the purity of their sample by thin-layer chromatography and performed a series of micro-bomb combustion experiments with a precision of about 0.4 percent. They obtained -16594 ± 66 kJ/mol (-3966 ± 16 kcal/mol).

A tabulation of the existing data is given in table 4.

TABLE 4. Comparison of results of prior investigations

ΔH_c° (25°C), kJ/mol	Investigators
- 16685 ± 166	Berthelot and Andre [6]
- 16656 ± 50	Bills, Cox, and Steel [7]
- 16594 ± 66	Paoli, Garrigues, and Patin [8]
- 16524.0 ± 3.9	This investigation

This work was supported, in part, by the Office of Standard Reference Materials, National Bureau of Standards.

6. References

- [1] Cohen, A., May, W., and Schaffer, R., Office of Standard Reference Materials, Institute for Materials Research, National Bureau of Standards, Washington, DC 20234 (June 6, 1974).
- [2] Prosen, E. J., and Johnson, W. H., in preparation.
- [3] Prosen, E. J., Chapter 6, Experimental Thermochemistry, F. D. Rossini, Ed. (Interscience Publishers, Inc., New York, N.Y., 1956).
- [4] Wagman, D. D., Evans, W. H., Halow, I., Bailey, S. M., and Schumm, R. H., Selected values of chemical thermodynamic properties, Nat. Bur. Stand. (U.S.), Tech. Note 270-3, 267 pages (Jan. 1968).
- [5] Handbook of Chemistry and Physics (Chemical Rubber Publishing Co., 2310 Superior Ave., N.E., Cleveland, OH., 1962).
- [6] Berthelot, M., and Andre, G., Ann. chim. phys. [7], **17**, 433 (1899).
- [7] Bills, C. E., Cox, W. M., Jr., and Steel, G. E., J. Biol. Chem. **84**, 655 (1929).
- [8] Paoli, D., Garrigues, J. C., and Patin, H., Compt. rend. **C268**, 780 (1969).

(Paper 79A3-854)